Single Extracellular Vesicle Characterization by Transmission Plasmonic Microscopy

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Label-free observation of transparent nanoparticles by far-field optical microscopy faces fundamental challenges in resolution and detection. They are difficult to detect because their ability to scatter light dramatically diminishes with decreasing size. Among various imaging techniques, those based on propagating surface plasmon resonance (SPR) and localized surface plasmon resonance (LSPR) have shown promise and respective limitations.

In this paper, we demonstrate ultra near-field index modulated PlAsmonic NanO-apeRture lAbel-free iMAging (PANORAMA) that addresses existing issues for both SPR and LSPR imaging techniques [1,2]. On one hand, PANORAMA can produce diffraction-limited lateral resolution free of the smearing effect in SPR imaging. PANORAMA also has higher surface sensitivity. PANORAMA addresses the sparse sampling issue in LSPR imaging by achieving dense sampling with a large imaging fill factor. The bright-field approach also provides much higher light throughput compared to dark-field microscopy, empowering higher imaging speed. Its system configuration is identical to a standard bright-field microscope using a trans- illumination tungsten-halogen lamp and a camera without the need for laser, LED, or interferometric detection. Therefore, PANORAMA is readily implementable on any existing commercial microscopes.

We have experimentally demonstrated that PANORAMA can image and size single polystyrene nanoparticle in water down to 25 nm, count individual nanoparticles within a sub-diffraction limit cluster, and dynamically monitor single nanoparticle approaching the plasmonic surface down to the millisecond timescale. The extrapolated size limit of detection is expected to reach sub-10 nm. The imaging speed is expected to be much higher with high-speed cameras. PANORAMA would provide new capabilities in label-free imaging and single nanoparticle analysis. Molecular-specific imaging has been demonstrated with surface functionalized plasmonic substrates for single biological nanoparticle analysis including extracellular vesicles (e.g., exosomes), an emerging cancer biomarker [3,4].

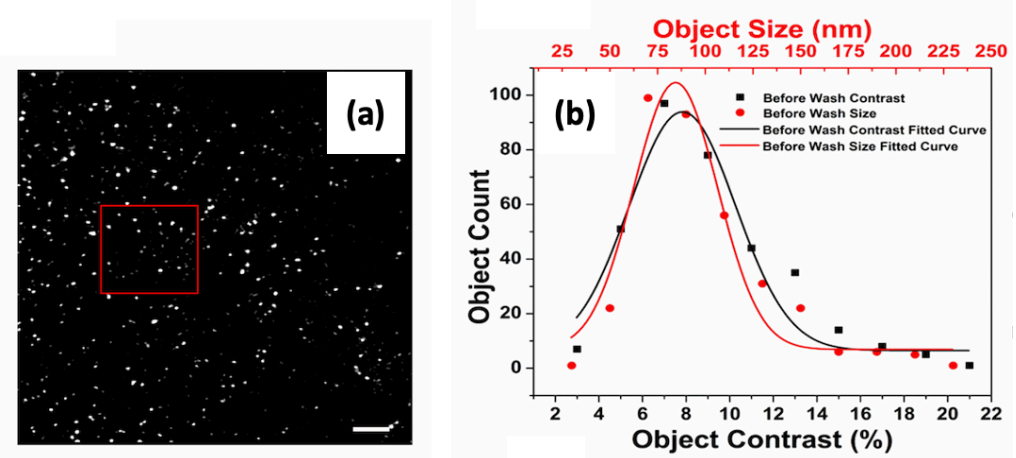

Figure 1: (a) PANORAMA image of exosomes on a nanoplasmonic substrate. (b) Histogram of exosome optical contrast and corresponding calibrated size.

References:

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